Toxicological Studies on the Effect of some Agricultural and Wild Plants Extract as Insecticidal Agent on the Common House Mosquito, *Culex pipiens* in Bisha Region, Saudi Arabia

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ABSTRACT

*Culex pipiens* plays a crucial role in the transmission of many vector-borne pathogens infecting humans, livestock and affecting wildlife. The present study was conducted in Laboratory of Biology Department, Faculty of Science, Bisha University, KSA, to evaluate the insecticidal activities of aqueous, ethanolic, and acetone extracts of each of nine selected wild plants, *Calotropis procera*, *Withania somnifera*, *Citrullus colocynthis*, *Mentha longifolia*, *Datura innoxia*, *Ziziphus spina christi*, *Salvadora persica*, *Aerva javanica*, and *Punica granatum* against larvae of *Cx. pipiens* under controlled laboratory conditions (water temperature ±2°C, 12:12 h photoperiod). After calculating the mortality percentages among treated larvae, LC₅₀ values could be arranged in an ascending order as follows: acetone extract < ethanolic extract < aqueous extract of *C. procera*, *W. somnifera*, *C. colocynthis*, *M. longifolia*, *D. innoxia*, and *Z. spina-christi*, respectively. On calculating the synergistic ratio (SR), it is found that the value of SR is greater than one in all tested extracts, except of *Ziziphus spina* which was lower than one. The SR of aqueous extract of *Calotropis procera* with Triton x100 (1.39) was higher than another one.

INTRODUCTION

WHO has considered mosquitoes as "public enemy number one", since mosquitoes can transmit more diseases than other arthropods and adversely affect millions of people all over the world. They act as vectors of various diseases some of which millions of cases have been recorded as illnesses and deaths in humans and animals every year. Among these diseases, malaria, yellow fever, dengue, filariasis, and Rift Valley fever have been documented along endemic and epidemic areas in many countries (WHO, 1997; Lerdthusnee et al., 1995). Saudi Arabia covers the major part of vast Arabian Peninsula. In this context, the southwestern region of Saudi Arabia has unique topographic features and climatic conditions. Therefore, it was considered as the nucleus of the first protected area for a large size of diverse biota, animals and plants. Most observations on urban and sub-urban, as well as rural areas of the southwestern region, have regarded mosquito fauna very limited, except those reported by some authors (Buttiker, 1979, 1981; Zahar, 1973; Hawking, 1973; Wills et al., 1985; Harbach, 1988; Abdullah and Merdan, 1995).
For preventing or minimizing the mosquito-borne diseases, as well as to improve the environment quality and public health, the mosquito control has been as a prerequisite need. The usual measure for mosquito control is the application of conventional insecticides, such as organochlorines and organophosphates (Poopathi and Kishore, 2006; Ghosh et al., 2012). Unfortunately, these insecticides are not successful agents owing to various human hazards, as well as numerous technical, operational, ecological, and economic problems. In other words, use of many conventional insecticides for mosquito control has been limited due to the high costs of these insecticides, concern for environmental sustainability, hazards against human health and other non-target organisms, non-biodegradable nature in the ecosystem, beside the increasing development of insecticide resistance on a global scale (Benelli et al., 2016; Schorkopf et al., 2016).

One of the effective alternatives of insecticides is the searching for natural materials in the floral biodiversity, as a simple and sustainable method of mosquito control. Further, unlike conventional insecticides, which are based on a single active ingredient, plant-derived active materials usually contain botanical blends of compounds which act concertedly on both behavioral and physiological processes (Ghosh et al., 2012; Dinesh et al., 2014; Deepalakshmi and Jeyabalan, 2017).

For the continued effective vector control management, identifying efficient bio-insecticides that adaptive to the ecological systems is necessary. Botanicals have wide range of insecticidal properties and thus they can be used as a safe weapon to fight against mosquito-borne diseases, since they have been reported environmentally safe, less hazardous to non-target biota, simple, inexpensive, and more suitable for the developing countries (Soliman and El-Sherif, 1995; El-Bokl and Moawed, 1997; Shoukry and Hussein, 1998, Massoud and Labib, 2000; Mohammed and Hafez, 2000; Mohammed et al., 2003).

Among mosquitoes, the common house mosquito, Culex pipiens, is the most common and widely distributed species in Southwestern Saudi Arabia. Culex pipiens plays a crucial role in the transmission of many vector-borne pathogens infecting humans, livestock and affecting wildlife including viruses (Engler et al., 2013), protozoan parasites (Santiago-Alarcon et al., 2012) and Metazoan parasites (Morchón et al., 2007). Some authors reported that adult females of C. pipiens feed mainly on birds, but others (Muñoz et al., 2012; Martínez-de et al., 2013; Martínez-de et al., 2015) demonstrated that mammals represent also an important source of their blood meals in some populations. Therefore, the present study was conducted to evaluate the insecticidal activities of different extracts of nine selected wild plants, as biorational agents, against C. pipiens. Also, a synergistic action of TritonX100 on the susceptibility of C. pipiens larvae to wild plant extracts was investigated in order to improve the properties of the tested materials.

**MATERIALS AND METHODS**

1. Study Area:

   Bisha is a region in the south-western Saudi Arabian province, 'Asir'. It is located at 20°0'0"N 42°36'0"E. Bisha includes about 58 towns and 240 villages spreading out on both sides of Bisha Valley. It stands at an altitude of approximately 610 meters (2,000 ft.) above sea level. Southwestern region is located between latitude 17° 30--21° 00'N and Longitude 41° 30--44° 30E. It is entirely different from the rest of the Kingdom. Asir highland receives a variable seasonal rainfall but more than the rest of the Kingdom (300-500 mm/year as compared to 50-100 mm/year elsewhere).
According to the characteristic of the study area and its environmentally biotic and abiotic factors, it could be considered a unique study area for mosquito ecology and distribution. The area also includes variable water resources for all types of mosquito breeding sites.

2. Plant Collection:

Leaf and seed samples were collected from the valley in Bisha region during the period August 2017-March 2018. The leaf and seed samples were packaged and transported to Laboratory of Biology Department, Faculty of Science, Bisha University, for processing extraction and bioassays. The collected plants were systematically identified by the co-worker Dr. T. Dahan, Bisha University. The collected plants can be described as follows (see Table 1 and Plate 1).

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Family</th>
<th>Common name</th>
<th>Plants part</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Calotropis procer&lt;i&gt;a</td>
<td>Asclepiadaceae</td>
<td>Milk weed</td>
<td>Leafs</td>
</tr>
<tr>
<td>2 Withania somnifera</td>
<td>Solanaceae</td>
<td>Winter cherry</td>
<td>Leafs</td>
</tr>
<tr>
<td>3 Citrullus colocynthis</td>
<td>Cucurbitaceae</td>
<td>Bitter apple fruit</td>
<td>Seeds</td>
</tr>
<tr>
<td>4 Mentha longifolia</td>
<td>Lamiaceae</td>
<td>wild mint</td>
<td>Leafs</td>
</tr>
<tr>
<td>5 Datura inoxia</td>
<td>Solanaceae</td>
<td>Indian apple</td>
<td>Leafs</td>
</tr>
<tr>
<td>6 Ziziphus spinachristi</td>
<td>Rhamnaceae</td>
<td>Jujube</td>
<td>Leafs</td>
</tr>
<tr>
<td>7 Salvadora perssica</td>
<td>Salvadoraceae</td>
<td>tooth brush</td>
<td>Leafs</td>
</tr>
<tr>
<td>8 Aerva javanica</td>
<td>Amaranthaceae</td>
<td>Aerva</td>
<td>Leafs</td>
</tr>
<tr>
<td>9 Punica granatum</td>
<td>Myrtales</td>
<td>the epic poem</td>
<td>Punica</td>
</tr>
</tbody>
</table>

Plate (1) Showing the picture of the collected plants
2.1. Calotropis procera: Common names include apple of Sodom, Sodom apple, stabragh king’s crown, rubber bush, or rubber tree (Morris et al., 1964; Adebayo et al., 2015). It is a flowering plant belonging to family Apocynaceae that is native to North Africa, Tropical Africa, Western Asia, South Asia, and Indochina. The green globes are hollow but the flesh globes contain a toxic and extremely bitter milky sap that turns into a gluey coating resistant to soap.

2.2. Withania somnifera: Common names include ashwagandha, Indian ginseng, poison gooseberry, and winter cherry (Stearn, 1995). It belongs to the family Solanaceae (nightshade family). However, several other species in this genus are morphologically similar to the selected species. Although those species are commonly used as medicinal herbs in Ayurvedic medicine, there is no high-quality clinical evidence that they have biological effects on insects.

2.3. Citrullus colocynthis: Common names include colocynth, bitter apple, bitter cucumber, desert gourd, egusi, vine of Sodom, and wild gourd (Gurudeeban et al., 2010). It is a desert vine plant growing in sandy arid soils. It is native to the Mediterranean Basin and Asia, and is distributed among the west coast of northern Africa, eastward through the Sahara, Egypt until India, reaching to the north coast of the Mediterranean and the Caspian Seas. In addition, it grows in southern European countries, as Spain, and on islands of the Grecian archipelago. Naturally, it is an annual or a perennial plant in Indian arid zones and has a great survival rate under extreme xeric conditions (Gurudeeban et al., 2010). The seeds are grey and 5 mm long by 3 mm wide.

2.4. Mentha longifolia: Common name is horsemint. It is a species in the genus Mentha (mint), native to Europe, western and central Asia (east to Nepal and the far west of China), and Northern and Southern (but not tropical) Africa. It is a very variable herbaceous perennial plant with a peppermint-scented aroma. It spreads via rhizomes to form clonal colonies (Huxley, 1992).

2.5. Datura innoxia: Common names include prickly burr, recurved thorn-apple, downy thorn apple, Indian apple, lovace, moonflower, nacazcul, toloatzin, toluache or toloache (Preissel et al., 2002). It is an annual shrubby plant whose height reaches 0.6-1.5 meters. Stems and leaves are covered with short and soft grayish hairs, giving the whole plant a grayish appearance. The elliptic smooth-edged leaves have pinnate venation. Fruit is an egg-shaped spiny capsule, about 5 cm in diameter. Fruit splits open when ripe, dispensing the seeds.

2.6. Ziziphus spina-christi: Common name is Christ's thorn jujube. It is an evergreen plant, native to Northern and tropical Africa, Southern and Western Asia. It is native to the countries of Chad, Djibouti, Eritrea, Ethiopia, Kenya, Libya, Mali, Mauritania, Nigeria, Pakistan, Senegal, Somalia, Tunisia, Turkey and Zimbabwe (The Plant List, 2016).

2.7. Salvadora persica: Common names include arak, Galenia asiatica, meswak, peelu, pilu, S. indica, or toothbrush tree, mustard tree, and mustard bush. It is a species of Salvadora and has antiurolithiatic properties, and used for centuries as a natural toothbrush. Its fibrous branches have been recommended by the World Health Organization for oral hygiene use (Batwa, Mohammed; et al., 2006; Araya, Yoseph 2008).

2.8. Aerva javanica: Common names include the kapok bush or desert cotton (Willis, 1985). It is a species of plant in the Amaranthaceae family. It has a native distribution incorporating much of Africa (including Madagascar), and the southwest and south of Asia, and it has become adventitious in northern Australia. The
plant is herbaceous, multi-stemmed and soft-wooded. It bears broad leaves. It often has an erect habit and grows to a height of about 1.6 meters. In Western Australia, this plant tends to grow in sandy soils, especially along drainage lines.

2.9. *Punica granatum*: Common name is pomegranate. It is a fruit-bearing deciduous shrub or small tree in the family Lythraceae growing to 5-8 m tall. It has been cultivated since ancient times throughout the Mediterranean region. Today, it is widely cultivated throughout the Middle East and Caucasus region, north and tropical Africa, South Asia. In the 20th and 21st centuries, it has become more common in markets of Europe and the Western Hemisphere (Morton, 1987; Stover and Mercure, 2007).

3. **Plant Extraction Procedure:**

The collected plant materials (leaves or seeds) had been thoroughly washed to avoid dust and dirt. These washed materials were then kept under shade in the laboratory for drying. Dried parts of the previously mentioned plants were cut into small pieces and ground by an electric grinder. Hundred grams of the powdered materials of each plant were exhaustively extracted with water, absolute ethanol and acetone using Soxhlet extraction technique for 4-6 h. After cooling to room temperature, the resultant extract was concentrated and stored at −20 °C until the larvicidal bioassay.

4. **Mosquito Collection:**

Monthly collections of mosquito larvae (Augusts 2017-March 2018) were made from selected breeding places in the Bisha area (Bisha valley, Al Hazamy, and Behind Bisha Dam). These places were variable and ranged from permanent ones to occasional water collections, which include irrigation wells and canals, seepage, surface water collections, drainage water, and stagnant water. Collection was carried out by sweeping the water surface with the long-handled larval net (WHO, 1975). In small water collections, another larval net was used with the iron ring of 10 cm diameter. During collection, the aquatic stages were washed into the nylon sieve which was then inverted and washed out in a white enamel bowl containing clear distilled non-chlorinated water. All immature mosquito stages were picked up by a pipette and transferred into a plastic bag. All samples were transported to the laboratory in thermos box. At the laboratory, pupae and 4th instar larvae were isolated, each in separate vials containing small amount of breeding site water and covered until the adult emergence. Early larval instars were transferred to breeding enamel bowls, fed tropical fish food (Tetramin) and kept at 27+/-1°C. These instars were observed daily until moulting into the 4th instar.

5. **Culturing of Tested Mosquito:**

The collected *C. pipiens* larvae from Bisha Valley were used to establish a culture in the laboratory under controlled conditions (water temperature 28 ± 2 °C, 12:12 h photoperiod). The larvae were reared in large plastic pans (37 X 31 X 6 cm) containing distilled water. Larval densities ranged 200–300/pan. Each pan was supplemented with the artificial diet Tetramin® fish meal (Tetra GmbH, Melle, Germany). Each of rearing water and diet had been replaced with fresh materials every two days. Pupae were kept in plastic cups and transferred into standard 30 × 30 × 30 cm rearing cages for adult emergence. The newly emerged adults were provided with 10% sucrose solution in a cotton pad within a Petri dish. Mosquito females were blood-fed on a pigeon 4–5 days post-emergence and provided with
oviposition plastic containers (11.5 cm in diameter and ~ 6.2 cm in depth) for egg laying. The egg collection was conducted 2–3 days after blood meal. These egg rafts were ready for the maintenance of the present culture.

6. Toxicological Bioassay:
A series of toxicological bioassay was carried out to evaluate the insecticidal activities of the plant extracts on the *C. pipiens* larvae. Fourth larval instars were used for this purpose. Toxicological bioassay of the selected extracts on tested mosquito larvae was carried out according to method described by Wright (1971) with some improvements. The bioassay was conducted using batches of 20 (*n* = 20) pre-fourth instar larvae of *C. pipiens* per beaker. Five replicates were used for each concentration. Five replicates of control larvae were also used beside the treated larvae. Treated and control larvae were fed on TetraMin® fish meal during the testing period. Larval mortality was observed at intervals of 24 h until the death of the last instar larvae or adult emergence. Death of larvae was checked if they remained irresponsible within a span of two minutes when gently probed with a pipette. The number of the dead larvae was calculated as average mortality percentage for each concentration relative to mortality of corresponding controls.

7. Synergistic Action of Triton x100:
Each of extract was mixed with an appropriate concentration (1 mL) of the synergist Triton x100 (0.01%) to obtain mortalities as described before. Then, data were analyzed by the probit analysis (Finney, 1971) and the synergistic ratio (SR) was calculated empirically according to Thangam and Kathiresan (1990, 1997).

\[
SR = \frac{LC_{50} \text{ of extract alone}}{LC_{50} \text{ of the mixture;}}
\]

value ≥ 1 indicating synergism;

≤ 1 indicating antagonism.

8. Statistical Analysis of Data:
The treatment was considered valid when it was more than 10% mortality in the control larvae (Hassan, 1989). Percent mortality was corrected using Abbot's formula (Abbott, 1925) when necessary. The lethal concentrations were deduced by extrapolation from the regression line obtained by Probit analysis (Finney, 1971). The *LC*\(_{50}\) and *LC*\(_{95}\) values were determined at their associated 95% confidence levels as well as their slope function, according to (Finney, 1971).

### RESULTS

The insecticidal activities of aqueous, ethanolic, and acetone extracts of nine tested plants (*C. procera*, *W. somnifera*, *C. colocynthis*, *M. longifolia*, *D. innoxia*, *Z. spina-christi*, *S. perssica*, *A. javanica*, and *P. granatum*) were evaluated against the 4\(^{th}\) instar larvae of the *Cx. pipiens* in the laboratory.

The results were presented in Tables (2, 3 & 4), and the regression lines were illustrated in Figures (1-6). The confidential limits of each of the tested nine plants were statistically calculated for *LC*\(_{50}\) and *LC*\(_{95}\) at *P* = 0.05. Extracts of these nine plants showed different toxicities except for the last three plants, *S. perssica*, *A. javanica*, and *P. granatum* which exhibited very slight toxic effects. The *LC*\(_{50}\) values of aqueous, ethanolic and acetone extracts were determined in 17.29, 22.22, & 25.01 ppm, respectively, of *C. procera*; 46.73, 54.11 & 59.30 ppm, respectively, of *W. somnifera*; 64.76, 84.50 & 106.81 ppm, respectively, of *C. colocynthis*; 380.38, 429.69 & 553.56 ppm, respectively, of *M. longifolia*; 560.40, 622.70 & 657.84 ppm, respectively, of *D. innoxia*; and 1667.04, 1789.17 & 1881.85 ppm, respectively, of *Z.
spina-christi. Therefore, the toxicity of the tested plant extracts based on LC\textsubscript{50} values which could be arranged in an ascending order as follows: acetone extract < ethanolic extract < aqueous extract of C. procera, W. somnifera, C. colocynthis, M. longifolia, D. innoxia, and Z. spina-christi. In addition, toxicities of the tested extracts were found in a dose-dependent course. Besides lethality of high doses, the extracts remarkably accelerated the growth of larvae into pupae.

1. Larvicidal Activity of Aqueous Extracts Against C. pipiens Larvae:

After treatment of mosquito larvae with aqueous extracts of the tested plant species, data of toxicity had been distributed in Table 2 and delineated in the regression lines of Figures 1 & 2. LC\textsubscript{50} values of aqueous extracts of C. procera, W. somnifera, C. colocynthis, M. longifolia, D. innoxia, and Z. spina-christi were recorded as 17.29, 46.73, 64.76, 380.40 and 1667.04 ppm, respectively. As shown in the present data, there were slight differences in potency of extracts of two plants, C. procera and W. somnifera. Also, aqueous extracts of these two plants were found in parallel regression lines. This might suggest that these extracts have the same mode of action against the tested mosquito larvae.

Data of the synergistic action resulting from adding 1 mL of Triton x100 (0.01%) to the same concentrations of aqueous extracts of all plants had been arranged in Table 2. These data revealed a considerable increase in LC\textsubscript{50} and LC\textsubscript{95} values, in comparison with those of each extract of C. procera, W. somnifera, C. colocynthis, M. longifolia, D. innoxia, and Z. spina-christi alone. By calculating the synergistic ratio (SR), SR was greater than one in all plants except of Z. spina-christi which was lower than one. SR of aqueous extract of C. procera with Triton x100 (1.39) was higher than another one.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Part of plant</th>
<th>Extraction</th>
<th>LC\textsubscript{50} (Co. Limits)</th>
<th>LC\textsubscript{95} (Co. Limits)</th>
<th>Slope function</th>
<th>S.R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calotrops procera</td>
<td>Leaves</td>
<td>Aqueous extract</td>
<td>17.29 (18.96-15.76)</td>
<td>43.87 (52.45-36.68)</td>
<td>4.072±0.109</td>
<td>1.393</td>
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<td></td>
<td>Aqueous + Triton x100</td>
<td>12.41 (13.88-11.09)</td>
<td>43.51 (54.83-34.68)</td>
<td>3.019±0.065</td>
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<tr>
<td>Withania somnifera</td>
<td>Leaves</td>
<td>Aqueous extract</td>
<td>46.73 (50.89-42.89)</td>
<td>118.86 (139.31-101.48)</td>
<td>4.056±0.126</td>
<td>1.097</td>
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<td>Aqueous + Triton x100</td>
<td>42.56 (46.47-38.97)</td>
<td>106.35 (123.50-91.64)</td>
<td>4.136±0.128</td>
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<tr>
<td>Citrullus colocynthis</td>
<td>seeds</td>
<td>Aqueous extract</td>
<td>64.76 (70.85-59.19)</td>
<td>157.39 (186.46-132.57)</td>
<td>4.264±0.119</td>
<td>1.278</td>
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<td>Aqueous + Triton x100</td>
<td>50.64 (56.07-45.73)</td>
<td>151.65 (185.04-124.46)</td>
<td>3.453±0.786</td>
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<tr>
<td>Mentha longifolia</td>
<td>Leaves</td>
<td>Aqueous extract</td>
<td>380.38 (422.29-342.60)</td>
<td>1087.47 (1293.51-914.88)</td>
<td>3.605±0.116</td>
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<td>Aqueous + Triton x100</td>
<td>334.13 (373.71-298.70)</td>
<td>928.06 (1092.49-788.72)</td>
<td>3.707±0.127</td>
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<tr>
<td>Datura innoxia</td>
<td>Leaves</td>
<td>Aqueous extract</td>
<td>560.40 (602.82-520.95)</td>
<td>1254.63 (1449.29-1086.32)</td>
<td>4.699±0.166</td>
<td>1.118</td>
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<td>Aqueous + Triton x100</td>
<td>501.03 (541.07-463.93)</td>
<td>1148.85 (1321.40-999.03)</td>
<td>4.564±0.148</td>
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<tr>
<td>Ziziphus spina-christi</td>
<td>Leaves</td>
<td>Aqueous extract</td>
<td>1667.04 (1829.47-1494.64)</td>
<td>5127.46 (7108.45-3700.30)</td>
<td>3.370±0.1576</td>
<td>0.973</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aqueous + Triton x100</td>
<td>1711.76 (1906.75-1536.83)</td>
<td>5025.67 (6913.73-3654.74)</td>
<td>3.516±0.1736</td>
<td></td>
</tr>
</tbody>
</table>
2. Larvicidal Activity of Ethanolic Extracts Against *C. pipiens* Larvae:

After treatment of mosquito larvae with ethanolic extracts, data were represented in Table 3 and the regression lines were illustrated in Figures 3 & 4. LC$_{50}$ values of ethanolic extracts of *C. procera*, *W. somnifera*, *C. colocynthis*, *M. longifolia*, *D. innoxia*, and *Z. spina-christi* were 22.22, 54.11, 84.50, 429.69, 622.70, and 1789.17 ppm, respectively. Also, these data showed the presence of differences in potency of the tested extracts.

Data listed in Table 3 indicated a considerable increase in LC$_{50}$ and LC$_{95}$ values in comparison with those of each extract of *C. procera*, *W. somnifera*, *C. colocynthis*, *M. longifolia*, *D. innoxia*, and *Z. spina-christi* alone. On calculating SR, it was greater than one in all tests except of *Z. spina* which was lower than one. SR of ethanolic extract of *C. colocynthis* with Triton x100 (1.25) was higher than another one (Table 3).
**Table 3** Larvicidal activity of ethanolic extractions of some plants against *Culex pipiens* larvae

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Part of plant</th>
<th>Extraction</th>
<th>LC&lt;sub&gt;50&lt;/sub&gt; (Co. Limits)</th>
<th>LC&lt;sub&gt;95&lt;/sub&gt; (Co. Limits)</th>
<th>Slope function</th>
<th>S.R</th>
</tr>
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<tbody>
<tr>
<td><em>Calotropis procera</em></td>
<td>Leaves</td>
<td>Ethanolic extract</td>
<td>22.22 (24.53-20.12)</td>
<td>63.64 (77.41-52.43)</td>
<td>3.59±8.375</td>
<td>1.22</td>
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<td>Ethanolic + Triton x100</td>
<td>18.11 (20.09-16.32)</td>
<td>55.54 (68.04-45.46)</td>
<td>3.38±7.708</td>
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<tr>
<td><em>Withania somnifera</em></td>
<td>Leaves</td>
<td>Ethanolic extract</td>
<td>54.11 (58.47-50.06)</td>
<td>129.55 (151.35-110.82)</td>
<td>4.33±0.145</td>
<td>1.19</td>
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<td>Ethanolic + Triton x100</td>
<td>45.31 (49.33-41.61)</td>
<td>112.64 (131.07-96.86)</td>
<td>4.15±0.129</td>
<td></td>
</tr>
<tr>
<td><em>Citrus colocynthis</em></td>
<td>Seeds</td>
<td>Ethanolic extract</td>
<td>84.50 (93.02-76.75)</td>
<td>229.98 (276.54-191.43)</td>
<td>3.78±0.143</td>
<td>1.25</td>
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<td></td>
<td></td>
<td>Ethanolic + Triton x100</td>
<td>67.41 (74.59-60.92)</td>
<td>190.01 (233.34-154.91)</td>
<td>3.65±0.102</td>
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<tr>
<td><em>Mentha longifolia</em></td>
<td>Leaves</td>
<td>Ethanolic extract</td>
<td>429.69 (469.70-393.67)</td>
<td>1069.93 (1279.93-940.34)</td>
<td>4.04±0.125</td>
<td>1.22</td>
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<td></td>
<td></td>
<td>Ethanolic + Triton x100</td>
<td>351.24 (389.44-316.755)</td>
<td>927.72 (1082.89-795.06)</td>
<td>3.89±0.129</td>
<td></td>
</tr>
<tr>
<td><em>Datura innoxia</em></td>
<td>Leaves</td>
<td>Ethanolic extract</td>
<td>622.70 (673.33-575.86)</td>
<td>1473.38 (1733.63-1252.84)</td>
<td>4.39±0.143</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethanolic + Triton x100</td>
<td>542.65 (591.98-497.42)</td>
<td>1449.78 (1733.64-1212.80)</td>
<td>3.85±0.113</td>
<td></td>
</tr>
<tr>
<td><em>Ziziphus spina-christi</em></td>
<td>Leaves</td>
<td>Ethanolic extract</td>
<td>1789.17 (2058.13-1555.60)</td>
<td>6870.86 (1068.15-4420.97)</td>
<td>2.81±0.134</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethanolic + Triton x100</td>
<td>1824.04 (2104.04-1581.57)</td>
<td>6980.03 (10921.28-4464.78)</td>
<td>2.82±0.137</td>
<td></td>
</tr>
</tbody>
</table>

Fig.3: Susceptibility of *Culex pipiens* larvae to ethanolic extractions of selected plants

Fig.4: Susceptibility of *Culex pipiens* larvae to ethanolic extractions of selected plants with Triton x100
3. Larvicidal Activity of Acetone Extracts Against *C. pipiens* Larvae:

After treatment of mosquito larvae with ethanolic extracts, data were assorted in Table 4 and the regression lines are diagrammatically presented in Figures 5 & 6. LC\(_{50}\) values for acetone extracts of *C. procera*, *W. somnifera*, *C. colocynthis*, *M. longifolia*, *D. innoxia*, and *Z. spina-christi* were found 25.01, 59.30, 106.81, 553.56, 657.84, and 1881.85 ppm, respectively. These data showed the presence of differences in potency of the tested extracts.

Data of the synergistic action resulting from adding 1 mL of Triton x100 (0.01%) with the same concentrations of acetone extract of all tested plants were distributed in Table (4). According to these data, there was a remarkable increase of LC\(_{50}\) and LC\(_{95}\) values, compared to those of each extract of *C. procera*, *W. somnifera*, *C. colocynthis*, *M. longifolia*, *D. innoxia*, and *Z. spina-christi* alone.

Results arranged in Tables 2, 3 &4 and graphically presented in Figures 1-6 indicated the susceptibility of *C. pipiens* to aqueous, ethanolic, and acetone extracts of the tested plants. Besides the potency of these extracts was less than chemical insecticides, they are more safer and conversion of plant material extraction to natural beneficial insecticide.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Part of plant</th>
<th>Extraction</th>
<th>LC(_{50}) (Co. Limits)</th>
<th>LC(_{95}) (Co. Limits)</th>
<th>Slope function</th>
<th>S.R</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Calotropis procera</em></td>
<td>Leafs</td>
<td>Acetonic extract</td>
<td>25.01 (27.28-22.94)</td>
<td>57.42 (67.28-49.06)</td>
<td>4.558 ±0.139</td>
<td>1.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetonic + Triton x100</td>
<td>20.30 (22.24-18.52)</td>
<td>49.76 (58.89-42.10)</td>
<td>4.224 ±0.126</td>
<td></td>
</tr>
<tr>
<td><em>Withania somnifera</em></td>
<td>Leafs</td>
<td>Acetonic extract</td>
<td>59.30 (63.82-55.10)</td>
<td>135.73 (158.70-116.15)</td>
<td>4.574 ±0.168</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetonic + Triton x100</td>
<td>49.32 (53.71-45.29)</td>
<td>128.58 (152.26-108.66)</td>
<td>3.952 ±0.124</td>
<td></td>
</tr>
<tr>
<td><em>Citrullus colocynthis</em></td>
<td>seeds</td>
<td>Acetonic extract</td>
<td>106.81 (116.93-97.57)</td>
<td>263.04 (311.09-222.56)</td>
<td>4.202 ±0.113</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetonic + Triton x100</td>
<td>84.25 (92.69-76.58)</td>
<td>221.59 (264.52-185.77)</td>
<td>3.916 ±0.105</td>
<td></td>
</tr>
<tr>
<td><em>Mentha longifolia</em></td>
<td>Leafs</td>
<td>Acetonic extract</td>
<td>553.56 (598.08-512.35)</td>
<td>1269.98 (1472.88-1095.28)</td>
<td>4.561 ±0.146</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetonic + Triton x100</td>
<td>485.60 (526.73-447.67)</td>
<td>1143.86 (1325.56-987.30)</td>
<td>4.420 ±0.137</td>
<td></td>
</tr>
<tr>
<td><em>Datura innoxia</em></td>
<td>Leafs</td>
<td>Acetonic extract</td>
<td>657.84 (715.45-605.85)</td>
<td>1539.34 (1781.15-1328.97)</td>
<td>4.458 ±0.121</td>
<td>1.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetonic + Triton x100</td>
<td>533.83 (586.17-486.15)</td>
<td>1416.50 (1669.69-1202.14)</td>
<td>3.881 ±0.507</td>
<td></td>
</tr>
<tr>
<td><em>Ziziphus spina-christi</em></td>
<td>Leafs</td>
<td>Acetonic extract</td>
<td>1881.85 (2138.23-1656.41)</td>
<td>5892.37 (8604.5-4037.34)</td>
<td>3.318 ±0.170</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetonic + Triton x100</td>
<td>1935.27 (2212.02-1693.37)</td>
<td>6121.41 (9080.52-4129.00)</td>
<td>3.289 ±0.182</td>
<td></td>
</tr>
</tbody>
</table>
Toxicological studies on the effect of some agricultural and wild plants extract as insecticidal agent

DISCUSSION

Economically, it is important to investigate the efficacy of plant extracts, as larvicidal agents, on the common house mosquito, *C. pipiens*. The principal criterion in the selection of these compounds was conversion of waste materials to useful one and their production in large scale was easy and costs less (Bakr and Al-Ghramh, 2014).

Huge number of reported research works had focused on the botanical extracts of the indigenous plants of Egypt and their toxic effects on different insect species (e.g., Soliman and El-Sherif, 1995; Messeha, 1997; El-Kassas, 2001; Attiaa, 2002, Mohamed et al., 2003, Kamel et al. 2005, Bakr et al., 2006, Mansour et al., 2011, El-Maghryab etal. 2012, Al-Zarog and El-Bassiouny, 2013; Elsiddig, 2015; Hosam et al., 2016; Abbas et al., 2017; Elhalawany and Dewidar, 2017; El-Rehawy, 2017, Khater, 2017; Wahba et al., 2017; Fouda et al., 2017; Bakr and Abd El-Bar, 2017; Bakr et al., 2017, 2018). In agreement with some of those reported results, the present study revealed differences in LC$_{50}$ and LC$_{95}$ values of the extracts of the nine tested plants species. Also, remarkable variations in the potency of tested extracts were recorded and can be attributed to the major constituents in each plant. They were found, also, to possess parallel regression lines of nearly equal slope values. This might suggest that these extracts have the same mode of action against the tested *Cx. pipiens* larvae (Busvine, 1971). Therefore, the difference in potency of these extracts may be referred to the quantity of the extracted materials rather than...
the quality of such materials (Mansour et al., 1996 and Bakr et al., 2006).

In the current investigation, the synergistic action resulting from adding 1 mL of Triton x100 (0.01%) to different concentrations of (aqueous, ethanolic, and acetone extracts) of the tested plants showed considerable increase in LC50 values. By estimation of SR, it was greater than one in all tested plants except of Z. spina which was lower than one. SR of acetone extract of C. colocynthis with Triton x100 (1.26) was higher than other one.

The larvicidal activity of all tested extracts on Cx. picipiens larvae was shown to increase greatly by adding Triton x100 which changes the surface tension of extract concentrations or dissolves the wax layer which covered the insects (Taylor and Schoof, 1967; Angus and Lutty, 1971; Mkhive and Gupta, 1985; Hussein, 1991; Husein et al., 2005; Kamel et al., 2005 and Mann and Koufman 2012).

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